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[COMMUNICATION]

Histochemistry of Yolk Formation in the Ovaries of the Tarnished Plant Bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae)

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ABSTRACT—The ovaries of *Lygus lineolaris* were studied using histochemical techniques. Three types of yolk bodies, YB I, YB II and YB III, are recognizable in vitellogenic oocytes. YB I granules were determined to be lipid by Sudan black B staining and YB II and YB III were protein/carbohydrate complexes based on bromphenol blue and periodic acid-Schiff staining. During early vitellogenesis, YB I and YB II are predominant, while in mature oocytes, YB I and YB III predominate suggesting that YB II may be a precursor for YB III. The germarium remains unchanged histochemically throughout the gonotropic cycle.

INTRODUCTION

Insect eggs contain a large amount of yolk which is incorporated during vitellogenesis. In most insects, yolk consists mainly of lipid and protein (with or without conjugated carbohydrates); and in some insects, glycogen deposits are also found. In many cases, yolk is of an extraovarian origin and the fat body is the most common site of storage and synthesis of yolk components [1–3]. In addition, the nurse cells in meroistic ovarioles and follicle cells may contribute some yolk material. Nurse cells may be associated with each developing oocyte as in polytrophic ovarioles or housed exclusively in the germarium as in telotrophic ovarioles common to Hemiptera. The development of nutritive cords in the latter type has made this an interesting developmental system to study. There have been a few histochemical studies of vitellogenesis in the Hemiptera (for example, *Acanthocephala,* Coreidae [4]; *Oncopeltus,* Lygaeidae [5–7]; *Gerris,* Gerridae [8]).

A recent study [9] showed that the gonotropic cycle in *Lygus lineolaris* requires seven days to be completed. The current paper reports of observations on the histochemical changes during vitellogenesis in *L. lineolaris*.

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MATERIALS AND METHODS

Insects were obtained and raised as described previously [9]. Virgin females aged 1-7 days were used throughout the study. Insects were anesthetized under CO_2 and dissected in insect Ringer [10]. Ovarioles with part of their associated lateral oviducts were removed, fixed and stored in 10% buffered formalin until use. Tissues used for detection of carbohydrate and protein were dehydrated through graded series of ethanol, passed successively through 1/2, 1/1, 2/1 Sorvall® embedding medium (E. I. Dupont Co. Newtown, Conn.)/absolute ethanol, infiltrated with three fresh changes of 12 hr each of embedding medium and embedded in the same at room temperature under nitrogen. Plastic sections (3–4 μm) were cut on a LKB ultratome using glass knives.

Periodic acid-Schiff (PAS) techinique with and without periodic acid oxidation was employed as a general test for carbohydrate [11]. Dimedone was used as a free aldehyde blocking agent [12]. To demonstrate glycogen deposits, plastic sections

were incubated in 0.5% diastase at 37°C for 30 min [13] followed by PAS staining. Control sections were similarly processed except for the enzyme incubation.

Treatment of sections with 0.1% bromphenol blue in 70% ethanol was used to demonstrate protein [13]. For detection of lipid, tissues previously fixed in 10% buffered formalin were washed overnight in water and passed through 5% and 10% gelatin for 2 hr each at 37°C in a vacuum oven. Tissues were then infiltrated with 25% gelatin overnight and embedded in the same at 4°C. Gelatin blocks with tissues were frozen with dry ice and 4 μm cryosections were cut on an IEC cryostat (IEC Co. Ltd., Needham Heights, Mass.). Sections were picked up on a warm glass slide, stained with Sudan black B in propylene glycol [14] and mounted in glycerin jelly.

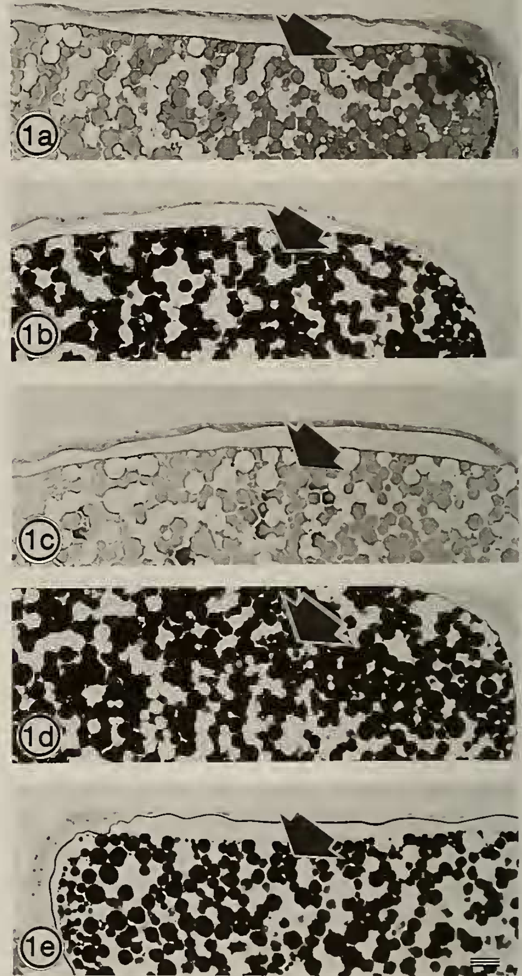
Tissue sections were examined under a Zeiss compound microscope and photographed with Panatomic X film (Kodak, 32 ASA).

OBSERVATIONS

Three types of yolk bodies, YB I, II and III are distinguishable in vitellogenic oocytes in *L. lineolaris*. Results from bromphenol blue and PAS staining suggested that YB II and III are protein carbohydrate complexes (Figs. 1, 10, 11 and 12). Combination of PAS staining with diastase treatment further confirmed that the carbohydrate moiety of YB II and III is not glycogen (Fig. 1). YB II is more intensely stained (dark blue) with bromphenol blue and is relatively smaller (ca. 2–8 μm) than YB III (pale blue and ca. 5–25 μm) (Fig. 12). YB I is not preserved in plastic sections but is stained bluish black with sudan black B in cryosections suggesting its lipoidal nature (Figs. 2 and 3).

Throughout the gonotropic cycle, neither nurse cells nor follicle cells were observed to have undergone any histochemical changes. PAS positive and bromphenol blue stained cellular inclusions were not observed in the trophic core and the nutritive cord (Figs. 4 and 5).

At early vitellogenesis, PAS positive flocculent material accumulates in the extraovarian space beneath the follicular epithelium (Fig. 6, 7 and 8). Small droplets of YB I and II appear at the periphery of the oocyte and gradually proceed into the central region of the oocyte (Fig. 9). Later in



Figs. 1. a-e. Sections of a mature egg of *L. lineolaris*. Arrow heads indicate protein/carbohydrate yolk stained by PAS (1b), bromphenol blue (1e), PAS treated with dimedone (1a), PAS without periodic acid oxidation (1c) and PAS with diastase digestion (1d). Clear areas between the protein/carbohydrate yolk are lipid yolk. Note the overall decrease in staining intensity of the section after blocking of free aldehyde groups with dimedone. Staining of protein/carbohydrate yolk in 1c is due to the counter stain picro-aniline blue, no PAS-positive reaction appears in these sections without going through periodic acid oxidation. Diastase treatment has no effect on PAS staining. All protein/carbohydrate yolk is stained with similar intensity by bromphenol blue. Plastic sections. (Bar=10 μm).

vitellogenesis, the relatively larger YB III begins to appear in the oocyte (Fig. 12). The end of vitellogenesis is indicated by the formation of a PAS positive vitelline membrane and the oocytes are filled up mainly with YB I and III (Fig. 1). This remains unchanged throughout choriogenesis and no histochemical changes are observed inside the mature oocytes even after ovulation.

DISCUSSION

The origin of lipid yolk in eggs of hemipteran insects has been a controversial subject. Three different origins for lipid yolk have been reported i.e., extraovarian, trophic core and follicle cells [5, 7, 15]. Previously we showed in *Lygus* that minute amounts of lipid from the trophic core enter the developing oocyte during vitellogenesis [9]. Current observations suggest that part of the lipid yolk in vitellogenic oocytes of *Lygus* originates from the hemolymph while the follicle cells do not contribute any lipid to the oocytes.

As in other insects, large amounts of carbohydrate (in association with protein) are incorporated into the vitellogenic oocytes of *L. lineolaris*. The role(s) of the carbohydrate component(s) in the yolk precursor in insects is still unknown. Whether it is for maintenance of protein structure requisite for yolk precursor recognition or has additional nutritive value for the embryos remains to be determined [16].

In *L. lineolaris*, two types of protein/carbohydrate yolk spheres may be differentiated in vitellogenic oocytes by bromphenol blue staining. Since bromphenol blue staining intensity reflects the number of dye binding groups [17], it is possible that conversion of YB II into YB III is a result of molecular modification of the yolk precursor after incorporation into the oocytes similar to

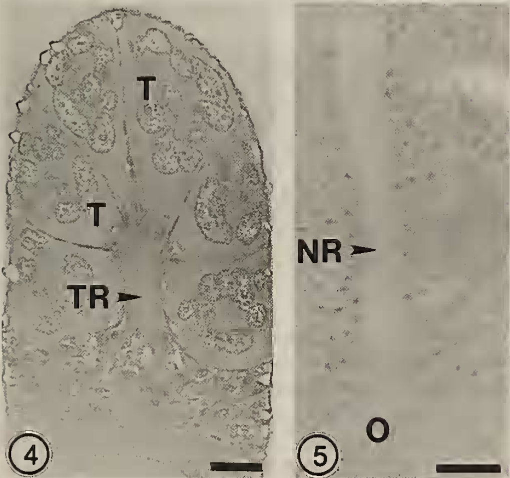
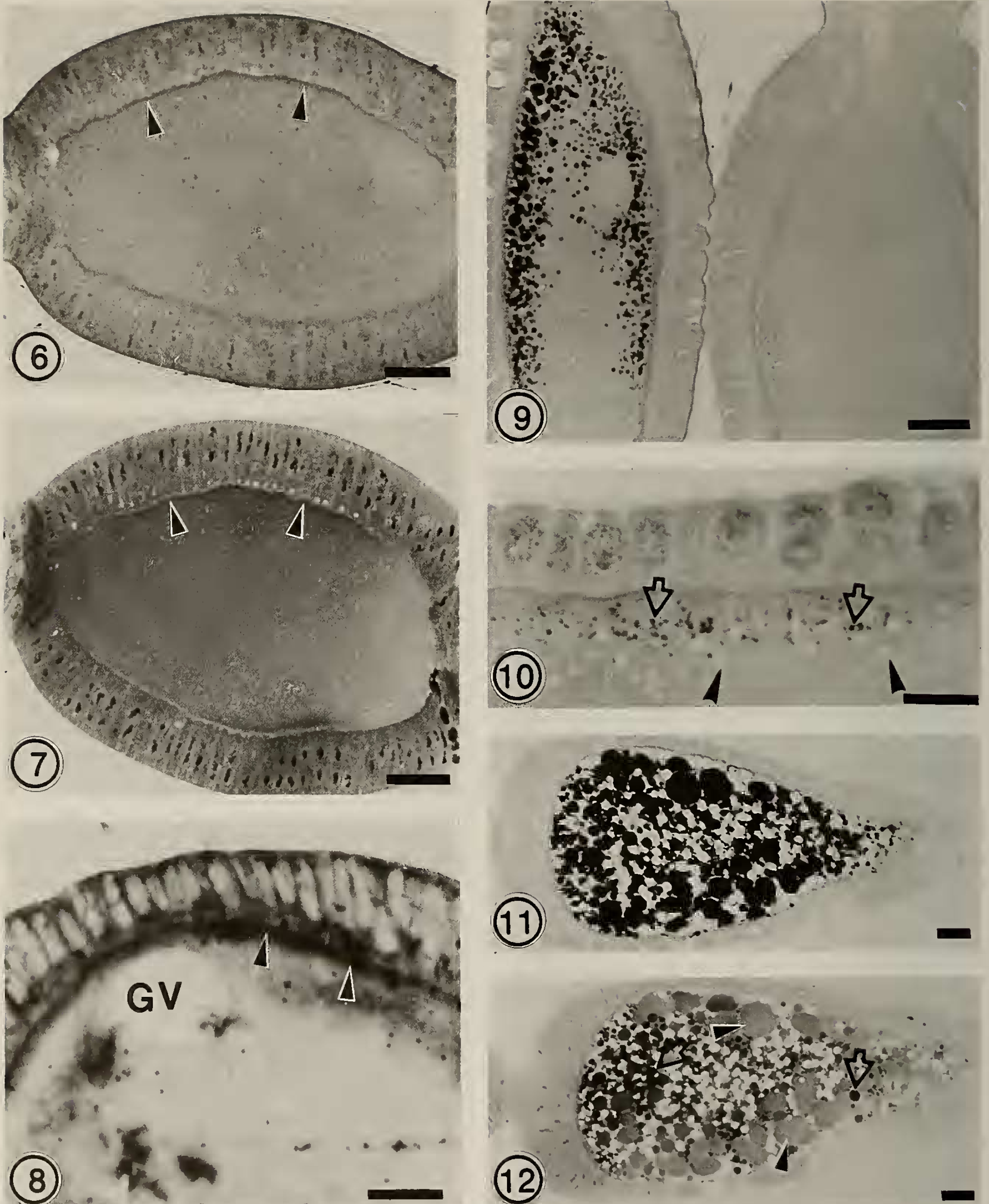


FIG. 2. Distribution of lipid yolk (arrow head) in a mature egg of *L. lineolaris*. Clear area around lipid yolk is due to protein/carbohydrate complexes which are very slightly stained. Cryosection, Sudan black B. (Bar=10 μm).

FIG. 3. Vitellogenic oocyte in *L. lineolaris* showing accumulation of lipid deposits on the oocyte surface (arrow) and also in the intercellular spaces (arrow heads) between follicle cells (FC). Cryosection, Sudan black B. (Bar=10 μm).

FIG. 4. Ovariole of *L. lineolaris* showing germarium that houses the nurse cells (T). Note the trophic core (TR) at the central region of the germarium which is bounded by nurse cells. The trophic core has a homogeneous overall staining and discrete cellular inclusions are not found. Plastic sections, PAS. (Bar=10 μm).

FIG. 5. The nutritive cord (NR) connects the trophic core (not shown) and the developing oocyte (O) throughout the gonotropic cycle until chorion formation. Note the uniform staining of the nutritive cord and the oocyte and also the absence of inclusions in the former. Plastic section, PAS. (Bar =5 μm).



FIGS. 6–8. Terminal oocyte at early vitellogenesis showing accumulation of yolk precursor (arrow heads) in the space between follicular epithelium and oocyte. This yolk precursor is PAS-positive (Fig. 6) (Bar = 10 μm) and stained dark blue by bromphenol blue (Fig. 7) (Bar = 10 μm). In cryosection, the yolk precursor is stained bluish black by Sudan black B (Fig. 8) (Bar = 5 μm). Note the large round germinal vesicle (GV) in the oocyte of Fig. 8.

FIG. 9. Terminal oocyte at early vitellogenesis (right) showing appearance of yolk droplets at the cortex of the oocyte. At later development, these yolk droplets coalesce and are seen to migrate into central core of the oocyte

that reported in cockroaches [18]. However, this is currently unknown in Hemiptera and further biochemical studies are necessary to validate the above hypothesis.

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(left). The PAS-positive flocculent material between follicular epithelium and oocyte is found to be more diffuse in oocyte at the right than the one at the left. Plastic section, PAS. (Bar = 10 μm).

Fig. 10. Higher magnification of an early vitellogenic oocyte indicates that both protein/carbohydrate yolk (open arrows) and lipid yolk (arrow heads) appear on the periphery of the oocyte. Plastic section, PAS. (Bar = 5 μm).

Figs. 11–12. Vitellogenic oocyte at later development is found to incorporate large amount of protein/carbohydrate yolk which is PAS-positive (Fig. 11). However, when stained with bromphenol blue (Fig. 12), this yolk can be distinguished into two types: YB II stained darker (open arrows) and ca. 2–8 μm in size and YB III stained lighter (arrow heads) and ca. 5–25 μm in size. Lipid yolk (YB I) is represented in these figures as clear areas around the protein/carbohydrate yolk. Plastic sections. (Bar = 10 μm).